

Characterization of Soluble and Colloidal-Phase Metal Complexes in River Water by Ultrafiltration. A Mass-Balance Approach

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■ Ultrafiltration, anodic stripping voltammetry, and atomic absorption spectrophotometry have been used to determine the distribution and degree of association of major trace metals with discrete size fractions of dissolved organic matter which passes initially through a 0.4- μm membrane filter. A novel mass-balance approach has been developed for fractionation of operationally defined, dissolved organic matter and organometallic complexes into well-defined molecular-size ranges. The highest concentrations of trace metals were found in intermediate molecular weight fractions (10^3 – 10^4) with no detectable free or labile metal as determined by anodic stripping voltammetry. Calcium and magnesium which were found predominantly in the lower molecular size ranges appear to have minimal impact on the extent of interaction of copper with dissolved organic matter. A residual complexation capacity for copper of $1.0 \pm 0.1 \mu\text{M}$ and relatively high pseudostability constant ($\log K = 10.4$) for copper complexation was observed.

Introduction

Water-quality studies have been focused primarily on the measurement of potentially toxic trace metals and anthropogenically derived organic substances and, in some cases, on their interactions in river water. However, naturally occurring organic matter has been implicated as an important factor governing the fate of trace metals (1–8). Adsorption, chelation, or entrapment of trace metals by dissolved or colloidal organic matter may affect biological availability, toxicity to microorganisms, and transport mechanisms in rivers. Some investigators (9–13) have suggested that complexation of micronutrients by soluble organic chelates can increase the physiological availability of trace metals to aquatic organisms when the ratio of organic matter to trace metal is reasonably high, while others (14–21) have shown inverse relationships between the complexation capacity of natural waters and relative toxicity of Cu and Cd to planktonic algae. These studies indicate that, in addition to total metal concentrations in soluble and particulate phases, it is necessary to know the form or species of the metal to reliably predict biological availability and ultimate fate in aquatic systems. Size fractionation by

ultrafiltration (UF) followed by atomic absorption spectrophotometry (AAS) and anodic stripping voltammetry (ASV) measurements of metal concentrations can be utilized to provide more detailed experimental information about trace-metal speciation in aquatic systems (5, 6, 16, 22).

Size fractionation by ultrafiltration has been employed previously to characterize naturally occurring organic matter (6, 23–29) and organometallic complexes (5, 16, 22, 30–41).

Advantages of the ultrafiltration method over gel permeation (24, 42), dialysis (37), ion exchange (6), and vapor pressure osmometry have been discussed adequately by other investigators. Disadvantages involved with the ultrafiltration method (22, 25–28, 30, 35, 37, 43) have been enumerated and discussed carefully.

With both the advantages and the disadvantages of UF clearly in mind, a new approach to UF size fractionation using a cascade procedure (35) and a system of mass-balance equations, which obviates the need for repeated washings (16) or excessive concentration (31), has been developed and applied to the problem of trace-metal speciation in the Mississippi and Minnesota Rivers.

Experimental Section

Apparatus. Millipore 90-mm High-Flux ultrafiltration cells and UMO5 (Amicon), PSAC, PTGC, PSED, and PTHK (Millipore Corp.) membranes were employed for ultrafiltration experiments. Atomic absorption measurements were made on a Perkin-Elmer Model 360 AAS equipped with a Heated Graphite Atomizer (HGA 2100) and a deuterium background corrector. A Princeton Applied Research polarographic analyzer Model 174A equipped with a Metrohm E410 HMD electrode and a Scientific Products Magne stir were used for anodic stripping voltammetry. An HP-7040A X-Y recorder was used to record scans. Dissolved organic carbon (DOC), total organic carbon (TOC), and total inorganic carbon (TIC) measurements were made with a Beckman 915-A carbon analyzer.

Sampling Procedure. Mississippi and Minnesota River water samples were obtained from locations designated as Bemidji (BEM, 1), Royalton (ROY, 2), St. Anthony Falls (SAF, 3), Inver Grove Heights IGH, 4), and Mendota Heights (MEN, 5). The Bemidji site was located near the headwaters of the Mississippi River, the Royalton site was located near prime agricultural land, and the St. Anthony Falls and Invergrove Heights sites were located near industrial/urban

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stretches of the river. Water samples were collected at mid-stream ~1 m from the surface by using a polyethylene (PE) bucket suspended from a bridge. Samples were stored in acid-cleaned polyethylene carboys and 2-l LPE bottles. Duplicate 100-mL samples were filtered through 0.40- μ m Nuclepore membranes using a Millipore polycarbonate filtration cell. Filtrates were acidified to pH 1.5 with ultrapure HNO₃ (Baker). Unfiltered water samples were collected in carbon-free glass bottles with Al-lined caps. All containers were rinsed 3 times with river water to preclude contamination. Temperature and pH were measured on site.

Laboratory Procedure. All water samples were stored at 4.0 °C before analysis. Bulk water samples were centrifuged continuously at 11 000 rpm by using a DuPont-Sorvall centrifuge with a KSB continuous-flow attachment. The resulting centrifugate was filtered through a 0.40- μ m membrane immediately before ultrafiltration. After pretreatment, a sample of known volume ($0.4 \leq v \leq 1.4$ L) was introduced into a well-cleaned UF cell, and the system was pressurized with nitrogen to 25 or 40 psig depending on the membrane to be used. The ultrafiltrate was collected in an acid-washed polyethylene bottle. When the retentate volume had been reduced to ~50% of the initial volume, the system was depressurized and the retentate was transferred to three different acid-washed bottles and stored for AAS, ASV, and DOC analysis. The AAS portion was acidified immediately. Volumes were determined with graduated cylinders. The ultrafiltrate from the above step was sequentially fractionated by using membranes with the next lower pore size until the smallest pore size was used. Volumes of both the retentate and the ultrafiltrate were recorded at each stage. Membranes were cleaned by soaking in purified water (Milli-Q/Milli RO 4 System, Millipore Corp.) at pH 2.4 for 24 h before use. Both membranes and cells were rinsed with 2 L of Milli-Q water (four cell volumes). The final 60 mL was collected for blank determinations.

Free and labile Cu, Cd, and Pb and complexation-capacity measurements for Cu were made by ASV in the direct-current mode with a plating potential of 0.8 V vs. SCE, a plating time of 150 s, a 30-s quiescent period, a scan rate of 20 mV/s, a current range of 0.2 μ A and a low pass filter time constant of 0.3 s. A CO₂/N₂ gas stream was used to maintain a H₂CO₃*/HCO₃⁻ buffering system. Carbon dioxide and N₂ (Matheson) was mixed by using Matheson rotometers No. 610 for CO₂ and No. 602 for N₂. After mixing, the mixture was deoxygenated with an acidic vanadate solution containing 2.3 g of V₂O₅, amalgamated Zn, and 12 M HCl in 250 mL of water. Standard Cu solutions (10⁻⁴–10⁻⁵ M) were made from AR copper wire dissolved in a small amount of concentrated HNO₃. NaClO₄ (G. F. Smith) was recrystallized and used as the inert electrolyte at a concentration of 30 mM.

Results

Specific analytical procedures for ultrafiltration were established expressly to maintain the integrity of the in situ metal-organic interactions. According to Buffle et al. (35), use of sequential ultrafiltration procedures minimizes the problems of inconsistent organic fractionations (25, 43), sorption (22, 37), and leakage of organic material when the retentate concentration factor is large (26, 27). To avoid a large concentration gradient, we have employed either partial separation or repetitive washing of the retentate with distilled water of fixed ionic strength. However, the latter procedure may result in irreversible alteration of the original metal-organic associations. In order to minimize the effect of solute enhancement by UF on metal-organic interactions and to resolve completely the distribution of organic matter, organometallic colloidal complexes, and microparticulate material according to molecular size, we developed a material-balance formalism.

Mass Balance - Sequential Ultrafiltration

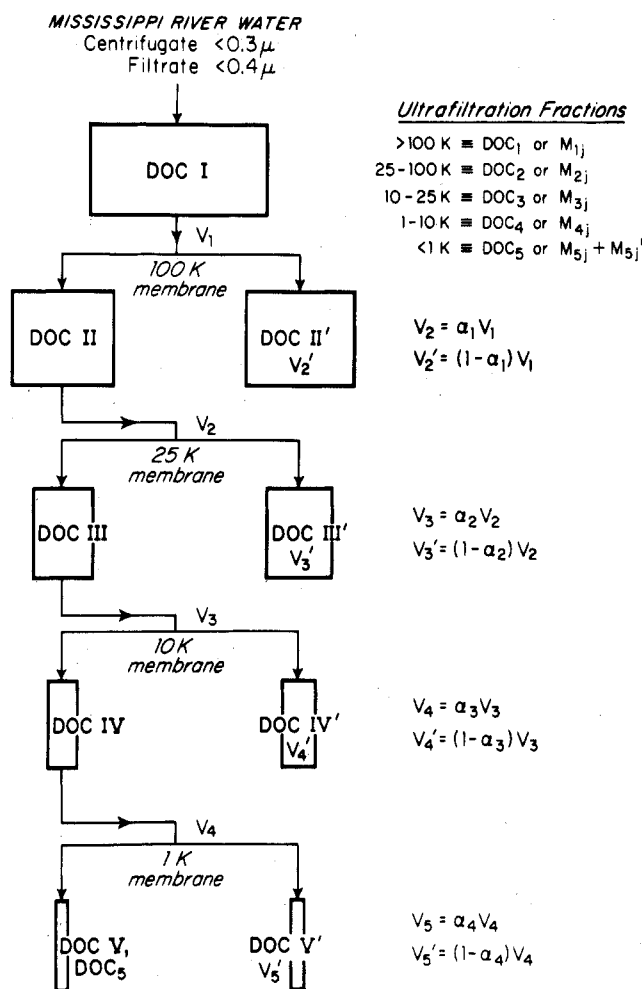


Figure 1. Flow diagram for sequential ultrafiltration procedure that illustrates the mass-balance approach and the successive volume reduction principle.

In sequential ultrafiltration successive volume reductions occur. This sequence is schematically illustrated in Figure 1. Since volume is reduced by 50% at each stage, both the filtrates and the retentates contain components of other molecular size fractions; consequently, those components contributing to each measured fraction must be taken into account in a mass balance.

The material-balance problem can be readily solved for organic carbon. The filtrates of each fraction filtered sequentially are missing organic matter of a size larger than the membrane cutoff. By measuring either the retentate or filtrate DOC in mg/L, one can determine the mass of DOC in each successive filtrate fraction if it is assumed that DOC of molecular weight less than the membrane cutoff passes in proportion to the volume, that the membrane behaves ideally as a molecular sieve at each cutoff, and that the volume is known. Given these conditions, a system of equations (Table I) for either retentate or filtrate fractions can be written which account for the total mass of organic carbon in the original (<0.40 μ m) sample. This system of five simultaneous equations has five unknowns for which an exact solution can be obtained. For DOC in mass units, the solution to the mass-balance problem is given in eq 1–5, where α_1 , α_2 , α_3 , and α_4 are successive volume reduction factors.

$$\text{DOC}_5 = (\text{DOC V})/(\alpha_1 \alpha_2 \alpha_3 \alpha_4) \quad (1)$$

$$\text{DOC}_4 = (\text{DOC IV})/(\alpha_1 \alpha_2 \alpha_3) - \text{DOC}_5 \quad (2)$$

$$\text{DOC}_3 = (\text{DOC III})/(\alpha_1\alpha_2) - \text{DOC}_4 - \text{DOC}_5 \quad (3)$$

$$\text{DOC}_2 = (\text{DOC II})/\alpha_1 - \text{DOC}_3 - \text{DOC}_4 - \text{DOC}_5 \quad (4)$$

$$\text{DOC}_1 = (\text{DOC I}) - \text{DOC}_2 - \text{DOC}_3 - \text{DOC}_4 - \text{DOC}_5 \quad (5)$$

Values for DOC I to DOC V in mass units are determined analytically by measurement of DOC (mg/L) in retentate or filtrate fractions and by knowing the total volume in each fraction. Alternatively the measured retentate masses may be used directly to solve the same problem. The solution presented in eq 1–5 can be expressed conveniently in the following series of equations for the filtrate fractions:

$$\text{DOC}_1 = (\text{DOC I}) - (\text{DOC II})/\alpha_1 \quad (6)$$

$$\text{DOC}_2 = (\text{DOC II})/\alpha_1 - (\text{DOC III})/(\alpha_1\alpha_2) \quad (7)$$

$$\text{DOC}_3 = (\text{DOC III})/(\alpha_1\alpha_2) - (\text{DOC IV})/(\alpha_1\alpha_2\alpha_3) \quad (8)$$

$$\text{DOC}_4 = (\text{DOC IV})/(\alpha_1\alpha_2\alpha_3) - (\text{DOC V})/(\alpha_1\alpha_2\alpha_3\alpha_4) \quad (9)$$

$$\text{DOC}_5 = (\text{DOC V})/(\alpha_1\alpha_2\alpha_3\alpha_4) \quad (10)$$

where $\text{DOC}_1 = >100\text{K}$ fraction, $\text{DOC}_2 = 25\text{--}100\text{K}$ fraction, $\text{DOC}_3 = 10\text{--}25\text{K}$ fraction, $\text{DOC}_4 = 1\text{--}10\text{K}$ fraction, and $\text{DOC}_5 = <1\text{K}$ fraction (K refers to molecular weight in multiples of 1000). A similar set of simultaneous equations (Table II) can be written for metal ions associated with the five different molecular-weight fractions, although in each case the summation of free aquated metal and low molecular weight inorganic complexes must be taken into account. Unfortunately, this results in five equations and six unknowns. For example, solution to the metal-retentate distribution is given in the following equations:

$$M_{1j} = \{M(\text{I})\} - M(\text{II})/\alpha_1 \quad (11)$$

Table I. Two Systems of Simultaneous Equations Used To Solve Sequential Ultrafiltration Mass Balance for Dissolved Organic Carbon in Mass Units

$\text{DOC I} = \text{DOC}_1 + \text{DOC}_2 + \text{DOC}_3 + \text{DOC}_4 + \text{DOC}_5$
$\text{DOC II}' (\text{retentate}) = \text{DOC}_1 + (1 - \alpha_1)[\text{DOC}_2 + \text{DOC}_3 + \text{DOC}_4 + \text{DOC}_5]$
$\text{DOC II} (\text{filtrate}) = \alpha_1[\text{DOC}_2 + \text{DOC}_3 + \text{DOC}_4 + \text{DOC}_5]$
$\text{DOC III}' (\text{retentate}) = \alpha_1\text{DOC}_2 + (1 - \alpha_2)\alpha_1[\text{DOC}_3 + \text{DOC}_4 + \text{DOC}_5]$
$\text{DOC III} (\text{filtrate}) = \alpha_1\alpha_2[\text{DOC}_3 + \text{DOC}_4 + \text{DOC}_5]$
$\text{DOC IV}' (\text{retentate}) = \alpha_1\alpha_2\text{DOC}_3 + (1 - \alpha_3)\alpha_1\alpha_2[\text{DOC}_4 + \text{DOC}_5]$
$\text{DOC IV} (\text{filtrate}) = \alpha_1\alpha_2\alpha_3[\text{DOC}_4 + \text{DOC}_5]$
$\text{DOC V}' (\text{retentate}) = \alpha_1\alpha_2\alpha_3\text{DOC}_4 + (1 - \alpha_4)\alpha_1\alpha_2\alpha_3\text{DOC}_5$
$\text{DOC V} (\text{filtrate}) = \alpha_1\alpha_2\alpha_3\alpha_4\text{DOC}_5$

Table II. System of Simultaneous Equations Used To Solve Sequential Ultrafiltration Mass Balance for Total <0.4 Metal

$$M(\text{I}) = \sum_{i=1}^5 M_{ij} + M_{5j}'$$

$$M(\text{II}) = \sum_{i=2}^5 \alpha_1(M_{ij} + M_{5j}')$$

$$M(\text{III}) = \sum_{i=3}^5 \alpha_1\alpha_2(M_{ij} + M_{5j}')$$

$$M(\text{IV}) = \sum_{i=4}^5 \alpha_1\alpha_2\alpha_3(M_{ij} + M_{5j}')$$

$$M(\text{V}) = \alpha_1\alpha_2\alpha_3\alpha_4(M_{ij} + M_{5j}')$$

$$M_{2j} = \{M(\text{II})/\alpha_1\} - M(\text{III})/(\alpha_1\alpha_2) \quad (12)$$

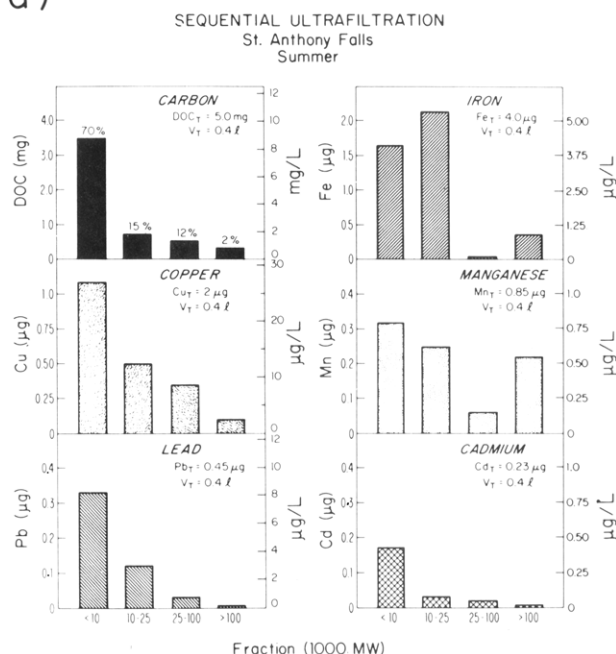
$$M_{3j} = \{M(\text{III})/(\alpha_1\alpha_2)\} - M(\text{IV})/(\alpha_1\alpha_2\alpha_3) \quad (13)$$

$$M_{4j} = \{M(\text{IV})/(\alpha_1\alpha_2\alpha_3)\} - M(\text{V})/(\alpha_1\alpha_2\alpha_3\alpha_4) \quad (14)$$

$$M_{5j} = \{M(\text{V})/(\alpha_1\alpha_2\alpha_3\alpha_4)\} - M_{5j}' \quad (15)$$

where M_{ij} is the mass of metal M_j in the i th molecular-weight fraction which is nominally associated with dissolved organic carbon or colloidal material, and M_{5j}' is the summation of free aquated metal and monomeric or polymeric inorganic complexes which because of their size should appear in the

(a)



(b)

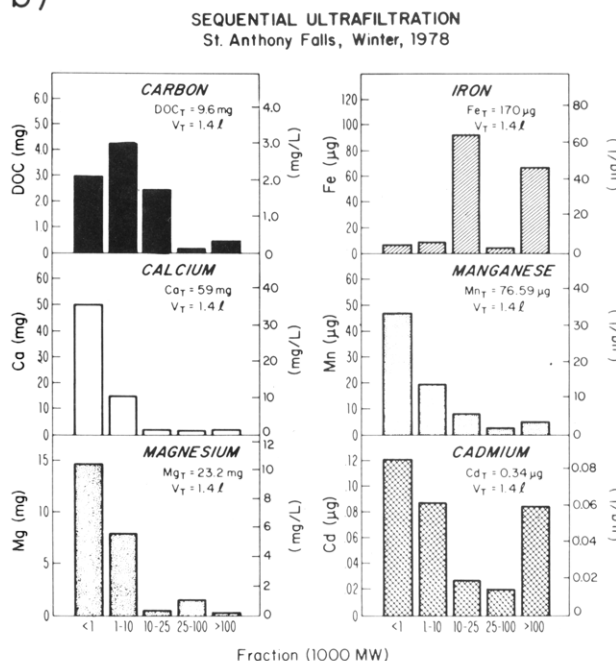


Figure 2. Results of sequential ultrafiltration separations for site 3 during summer 1977 (a) and for site 3 during winter 1978 (b). Concentrations for each fraction are given by M_{ij}/V_T with units of mg/L or $\mu\text{g/L}$. Additional water-quality data for site 3 averaged over 42 sampling dates are presented in ref 64.

smallest-size fraction. M_{5j}' can be estimated with ASV measurements for the special cases involving Cu, Zn, Pb, and Cd.

This general UF procedure assumes that, in the absence of metal associations in successive molecular fractions, as the total volume is reduced the mass of metal found in each successive fraction will be reduced proportionately. The reduction in mass of each metal in successive retentate fractions can be predicted by knowing the volume fraction factors and assuming a negligible adsorptive or desorptive interaction with the membrane or support surface. This simple hypothesis was tested with standard solutions of the appropriate metal salts in water at pH 2.5 and was shown to be valid.

Representative results of sequential ultrafiltration separations for SAF, summer and winter, are presented in bar graph fashion in Figure 2. Although flow and temperature conditions were typical of late summer for SAF, the terrestrial ecosystem conditions were autumnal. From these distributions some general observations can be made. The majority of organic carbon is found in less than 10K nominal molecular weight fractions. Trace metals such as Cu, Cd, Pb, and Mn are

found most frequently in size fractions which are less than 10K in molecular weight. However, a significant fraction of Fe, Mn, and Cd is found in higher molecular weight fractions. Sharp (44) has defined colloidal material to be in the size range of 2.8–12.0 nm and microparticulate material to be in the size range of 12.0–400 nm. Within the limits of these definitions, some of the Fe, Mn, and Cd in the higher molecular weight fractions may be colloidal or microparticulate in nature, suggesting that the oxides, oxyhydroxides, or surface-adsorbed species may be important factors controlling the speciation of these metals under certain conditions.

Calcium and magnesium were found to appear largely in the lowest molecular weight fractions, as shown in Figure 2b. When the 0.5K membrane was used, most of the Ca and Mg was found in the fraction which was less than 0.5K in nominal molecular weight.

Results of a spring 1978 sampling during a period of peak flow are presented in Figure 3a–d for sites 1, 2, 4, and 5. Analytical procedures for trace metals were complicated by the fact that concentrations of Cu, Pb, and Cd were extremely low. However, metal levels were consistent with an overall lower

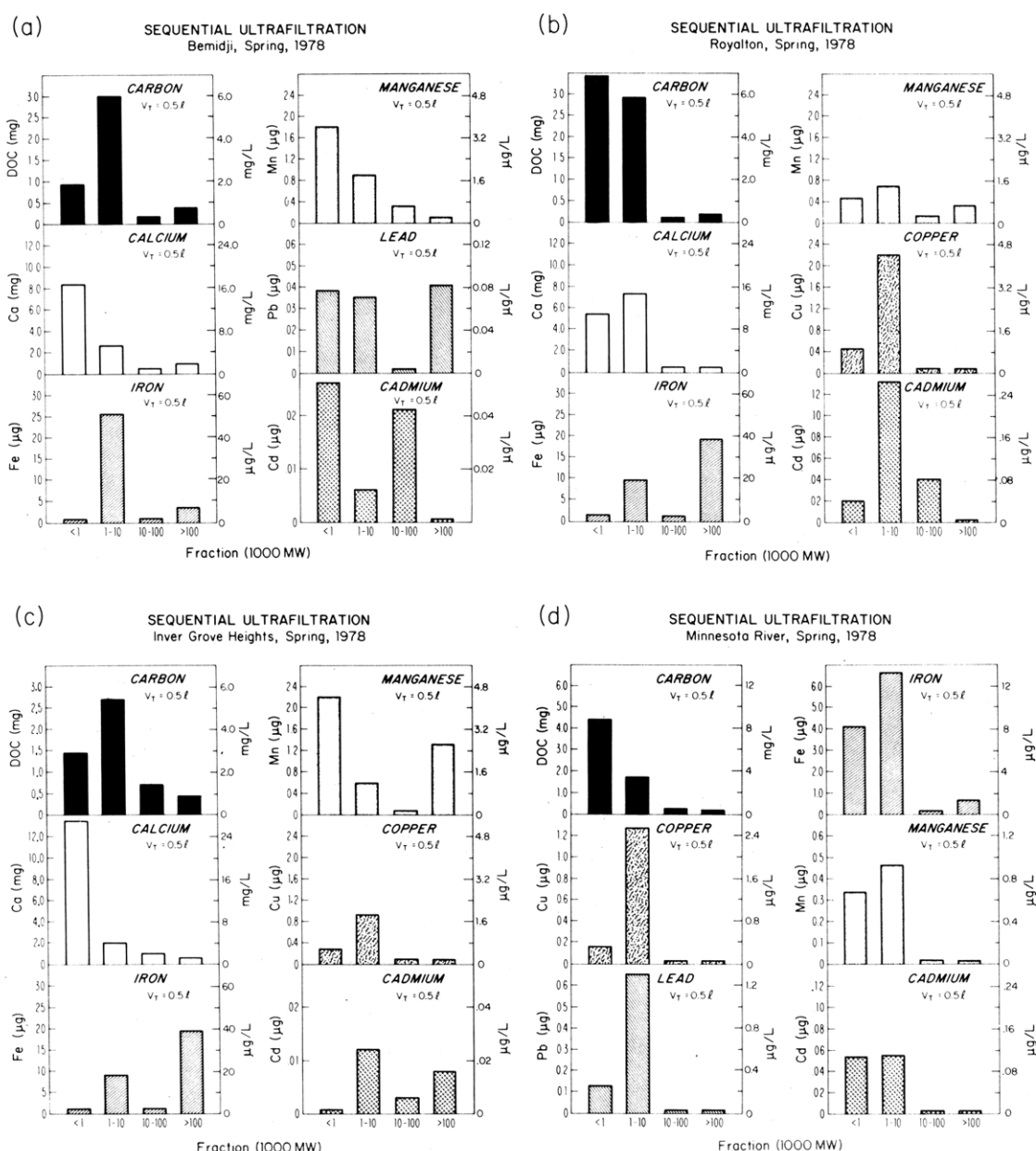


Figure 3. Results of sequential ultrafiltration separations for sites 1–5 during spring 1978. Concentrations for each fraction are given by M_{ij}/V_T with units of mg/L or µg/L. Additional water-quality data for sites 1–5 are presented in ref 64.

DOC level during spring runoff. Trends in metal distribution tended to approximate the distribution of organic carbon at each site with the major fraction of Cu and Cd found most frequently in the 1–10K fraction with the exception of Cd (<1K fraction) in the spring sample at Bemidji. With the exception of Fe at ROY and IGH, the majority of metals are found in the two lower molecular weight fractions. Anodic stripping voltammetry measurements for Cu, Cd, and Pb showed no detectable free or labile metal at the five study sites. These combined results suggest that organometallic complexes play an important role in trace-metal speciation in the upper Mississippi River.

Further evidence for possible organometallic interactions was provided by complexation-capacity measurements and pseudostability constant determinations. Complexation capacity or the residual complexing ability of a natural water sample was determined by complexometric titration of the <0.4 μM filtrate with soluble Cu(II) (39, 46–50) followed by ASV measurement of free and labile copper. Plots of ASV peak current vs. the cumulative Cu concentration were made, and the intersection of lines drawn from the initial and final linear response regions was defined as the residual complexation capacity (49–51). Complexation-capacity measurements for fall 1978 are listed in Table III. The average values for sites 1–4 were 0.88, 0.99, 0.90, and 1.08 μM , respectively.

Stability-constant determinations were based on the methods of Shuman and Woodward (49, 50) using ASV complexometric titration data. The conditional formation constant for the formation of a metal–ligand complex with an assumed stoichiometry of 1:1 can be determined from eq 16

$$K_{ML}' = \frac{C_M - (i_a/k)}{(i_a/k)(C_L - C_M + i_a/k)} \quad (16)$$

where C_M is the total analytical metal concentration, C_L is the total ligand concentration determined graphically, i_a is the anodic stripping peak current, and k is the slope of the upper portion of the titration curve. The numerator in eq 16 corresponds to the total complex concentration, and the denominator is the product of the ASV free-metal concentration, i_a/k , and the equilibrium ligand concentration, $(C_L - C_M + i_a/k)$. During the initial stages of titration when $C_L \gg C_M$, eq 16 can be reduced to

$$K_{ML}' = C_M / [(i_a/k)(C_L - C_M)] \quad (17)$$

Since samples were buffered by a continuous flow of CO_2 gas during titrations and because the samples had a high alkalinity, a slight correction for competitive complexation of Cu^{2+} by carbonate and hydroxy complexes was made according to the method proposed by Allen (52). This correction is based on the ASV current response when $C_M > C_L$ or, in the case of copper complexation, when $[\text{Cu}^{2+}]_T > [\text{L}]$. Essentially, this procedure provides a method of correcting for the impact of labile copper–inorganic complexes on the apparent equilibrium constant. In the absence of competitive ligands, electrochemically active copper is given by $[\text{Cu}^{2+}]_T = [\text{Cu}^{2+}] + [\text{Cu}(\text{OH})_n^{(2-n)+}]$. However, because of the constant level of $[\text{H}_2\text{CO}_3^*]$ and the high initial alkalinity of the river water

samples, the ASV response in the upper region of the titration curve will be affected also by the formation of carbonate complexes. In order to account for known labile copper–inorganic complexes and to determine a K that more closely represents the nonlabile complexes in equilibrium with the free metal, Allen (52) proposed that the free hexaquo copper ion was proportional to the total labile metal as follows:

$$[\text{Cu}^{2+}] \approx \alpha_0[\text{Cu}^{2+}]_T \quad (18)$$

where

$$\alpha_0 = (1 + \beta_1[\text{OH}^-] + \beta_2[\text{CO}_3^{2-}] + \beta_3[\text{CO}_3^{2-}]^2)^{-1} \quad (19)$$

and β_1 , β_2 , and β_3 are the formation constants for the monohydroxy, monocarbonate, and dicarbonate complexes, respectively. An essential assumption in this development is that CuL complexes are not ASV active. However, some metal–organic complexes may be electrochemically labile because of relatively rapid dissociation rates. Consequently, ASV measurements may underestimate the total extent of residual organic complexation.

From the pH, alkalinity, and $\text{p}K_a$ values for H_2CO_3^* , a value for α_0 can be readily obtained, when values for β_1 , β_2 , and β_3 of $10^{6.3}$, $10^{6.75}$, and $10^{9.92}$, respectively, are used (53). Substitution of eq 18 for $[\text{Cu}^{2+}]$ in eq 17 followed by rearrangement will give the following expression:

$$i_a = \frac{k}{\alpha_0 K_{ML}'} \left(\frac{C_M}{C_L - C_M} \right) \quad (20)$$

A linear regression fit of eq 20 was used to determine K_{ML}' for Cu where C_L was given by the complexation-capacity measurement assuming the formation of a 1:1 complex. Average pseudostability constants for copper are given in Table III. $\log K_{ML}'$ ranged from 9.7 to 10.7 for sites 1–4. In all determinations of K_{ML}' , the effect of α_0 on the magnitude of the constant was slight.

Discussion

Ultrafiltration procedures and methods for data reduction by mass-balance techniques developed as a part of this study have been shown to be useful for characterization of metal complexes by size fractionation. When ultrafiltration is coupled with ASV measurements for Cu, Cd, and Pb, the degree of metal association in discrete molecular-size fractions can be determined. Common UF problems such as membrane clogging, leakage, concentration polarization, ionic-strength changes, and excessive concentration, which may lead to an alteration of the actual distribution of complexes, are minimized with these procedures when the concentration factor is ≤ 2 . Flushing procedures with artificial waters of similar composition and ionic strength may effect changes in the degree of metal complexation by dilution or introduction of trace-metal contaminants. However, it should be pointed out that size fractionation by UF with these procedures is not problem free. Nominal molecular weight cutoffs are imprecise since the shape and degree of ionization of the molecule is an important factor in retention by or passage through a particular membrane. Ogura (26) has shown that cytochrome *c* ($M_r = 12.6\text{K}$) was 95% retained by a 100K membrane and that cobalamin ($M_r = 1.357\text{K}$) was 76% retained by a 0.5K membrane, although urea ($M_r = 0.06\text{K}$) was not retained by either membrane. Macko et al. (43) have shown that the degree of ionization of low molecular weight organic acids is critical to the degree of retention by membranes with higher molecular weight cutoffs. They have shown also that retention is partially a function of flow rate and ionic strength when Ca^{2+} , Mg^{2+} , and SO_4^{2-} are primary solution components. Charged sulfonated membranes, which were developed to avoid fouling problems, are known to retain relatively high concentrations of Ca^{2+} and Mg^{2+} (54). Flow-rate problems can be overcome

Table III. Complexation-Capacity and Stability-Constant Data Fall 1978

site	TOC, mg/L	pH	$\text{p}C_T^a$	CC, μM	$\log K_{ML}$
1	18.4	8.05	2.39	0.88	10.4
2	13.2	8.23	2.60	0.99	9.9
3	24.3	8.29	2.52	0.90	10.7
4	17.6	8.16	2.43	1.08	10.0

^a C_T = total carbonate concentration.

by passing sufficient water through new membranes until a constant flux has been obtained or alternatively by using scrupulously cleaned and washed used membranes which tend to have relatively constant fluxes (43). When metal distributions are of primary concern, results of the present study show that new well-washed membranes with constant fluxes are preferable to clean but used membranes.

Storage procedures are also important in UF experiments. Unacidified field samples should be filtered as soon as possible to avoid sorptive losses to LPE bottle walls (55), especially when low levels of Cd, Pb, and Cu are present initially.

Size fractionation of the dissolved components of Mississippi River water at five different sites has shown that in most cases Cu, Cd, and Pb mass distributions are similar to those of dissolved organic carbon and that these metals are most often found in the 1–10K nominal molecular weight fraction. On the other hand, Fe and Mn are frequently found in higher molecular weight fractions and their dominant mass peaks do not correlate well with the mass distribution of organic carbon. ASV measurements as summarized in Table III show, firstly, that there is no detectable free or labile Cu, Cd, or Pb at any of the five sampling sites, secondly, that the residual complexation capacity for Cu is high, and, thirdly, that the pseudostability constants for Cu complexation are moderately high (i.e., 10^{10}). Stability constants in the range of $10^{9.9}$ – $10^{10.7}$ are indicative of metal complexes with multidentate organic ligands (56–58) containing a mixture of amino, sulfhydryl, carboxylate, or hydroxy functional groups.

Results of this study agree in general with the observations of other investigators who have studied molecular size distributions and Cu stability constants in freshwaters. Bacinni and Sutter (59) found most Cu in a number of Swiss lakes to lie in the 1–10K nominal molecular weight fraction as determined by ultrafiltration and gel filtration methods. They concluded that copper was complexed primarily by organic ligands and that the conditional stability constant at pH 8.8 was $\log K = 11.0 \pm 0.2$ as determined by ion-exchange. Complexation capacities or the total ligand concentration, C_L , was determined to be $2.4 \pm 0.5 \mu\text{M}$. Tuschall and Brezonik (60) studied the size distribution of isolated proteinaceous matter from two freshwater lakes and determined that the organic nitrogen had an apparent molecular weight between 1K and 50K. The complexation capacity and conditional stability constants for copper were determined to be in the range of $0.8 \leq C_L \leq 1.6 \mu\text{M}$ and $6.2 \leq \log K_{\text{CuL}} \leq 7.1$, respectively. They concluded that soluble organic nitrogen of proteinaceous origin may be important in the complexation of heavy metals in natural waters.

Buffle and co-workers (35, 61) found roughly similar molecular size distributions with the bulk of DOC in the 1–10K size range and conditional stability constants of 10.8–11.6 for Cu in Zaire river water. van den Berg and Kramer (62) used a MnO_2 adsorption technique to determine pseudostability constants for some Canadian rivers and lakes that were found in the range $7.2 \leq \log K_{\text{CuL}} \leq 9.5$. Similarly large stability constants ($\log K_{\text{CuL}} = 10.0$) were reported by Sunda and Hanson (21) for Cu in the Neuse River.

Results of this study and those reported above suggest that Cu complexation in many waters is dominated by aquatic organics and that in many waters formation of carbonate complexes is of secondary importance. The magnitude of the observed stability constants for apparent 1:1 complexes indicates that the ligands involved are multidentate. Conditional stability constants for Cu complexes with bidentate ligands such as glycine are on the order of $10^{6.5}$ at pH 8. Stronger multidentate ligands containing a mixture of amino, sulfhydryl, and carboxylate groups more closely approximate the observed Cu complexation characteristics in natural waters. Macrocyclic ligands such as porphyrins and phthalocy-

anines in which bonding takes place through pyrrole nitrogens may also play an important role in metal chelation in natural waters. The work of Tuschall and Brezonik (60) is particularly relevant in terms of relating apparent Cu complex stabilities directly to nitrogen-containing proteinaceous material. Similarly, Bacinni and Sutter (59) conclude that Cu may be preferentially bound to natural molecules containing peptide chains in which the N-terminal end can form a tridentate ligand for copper in the pH region of natural waters. Alternatively they (59) have suggested that Cu may be bound in a folded molecule such as a chromophore.

The potential role of multiple organic nitrogen and sulfur functionalities is strengthened by the experimental observation made by Perdue (63) that aquatic fulvic material isolated from Oregon river water contained aromatic carboxylic acids with two-thirds of the phenolic hydroxyl groups in meta and para positions. Only phenolic acids with *ortho*-hydroxyl groups could participate in chelation via salicylate-like functional groups. The apparent strength of Cu complexes in natural waters cannot be ascribed to carboxylate ligands alone.

Conclusion

Ultrafiltration, anodic stripping voltammetry, and atomic absorption spectrophotometry have been used to determine the distribution and the degree of association of major trace metals with discrete size fractions of organic matter which pass initially through a $0.4\text{-}\mu\text{m}$ membrane filter. A novel mass-balance approach has been developed for fractionation of operationally defined, dissolved organic matter and trace-metal species into well-defined molecular size ranges. In order to minimize adverse interactions with membrane surfaces and to ensure the integrity of metal complexes, we maintained volume reduction in the retentate fractions near 50% for a sequential ultrafiltration procedure. With known volume reduction factors and measured concentrations in either retentate or filtrate fractions, a system of simultaneous equations has been developed where the number of independent variables is equal to the number of independent equations. Application of this procedure to Mississippi and Minnesota River water shows that the highest concentrations of trace metals are often found in intermediate molecular weight (1 000–10 000) ranges with no detectable free or labile metal as determined by ASV. Calcium and magnesium are found predominantly in lower molecular size ranges. They appear to have minimal impact on the apparent extent of interaction of other metals with dissolved organic matter. The Mississippi River water shows a moderate residual complexation capacity for copper of $1.0 \pm 0.1 \mu\text{M}$ and a relative high pseudostability constant of 10^{10} M^{-1} . These results suggest that complexation of trace metals such as Cu, Cd, and Pb may be dominated by aquatic organics and that formation of carbonate complexes is of secondary importance.

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Photochemical Ozone Formation in the Irradiation of Ambient Air Samples by Using a Mobile Smog Chamber

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Although many studies have been carried out on the irradiation of synthetic hydrocarbon-nitrogen oxide mixtures and of auto exhaust in regard to photochemical air pollution, few studies (1-5) have reported on the irradiation of samples of ambient polluted air. All previous studies (1-5) on the irradiation of atmospheric samples were essentially concerned with the photochemical decay of individual hydrocarbons and with trying to characterize the relative hydrocarbon reactivity. Although the formation of products, oxidant, formaldehyde,

and peroxyacetyl nitrate was also reported (3-5), no systematic characterization of ozone (oxidant) formation has been reported.

Previous studies (6-8) at the National Institute for Environmental Studies (NIES) have revealed that, in the hydrocarbon-excess region, the maximum ozone formed ultimately ($[O_3]_{max}$) from 1-olefin-nitrogen oxide mixtures is, in general, proportional to the square root of both the initial concentration of NO_x and the primary photodissociation rate of NO_2 ,